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Measuring Initial Enamel Erosion with Quantitative Light-Induced Fluorescence and Optical Coherence Tomography: An in vitro Validation Study

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Key Words
Diagnostics · Initial enamel erosion · Measurement · Optical coherence tomography · Quantitative light-induced fluorescence

Abstract
Background: Measurement of initial enamel erosion is currently limited to in vitro methods. Optical coherence tomography (OCT) and quantitative light-induced fluorescence (QLF) have been used clinically to study advanced erosion. Little is known about their potential on initial enamel erosion. ***Objectives:*** To evaluate the sensitivity of QLF and OCT in detecting initial dental erosion in vitro. ***Methods:*** 12 human incisors were embedded in resin except for a window on the buccal surface. Bonding agent was applied to half of the window, creating an exposed and non-exposed area. Baseline measurements were taken with QLF, OCT and surface microhardness. Samples were immersed in orange juice for 60 min and measurements taken stepwise every 10 min. QLF was used to compare the loss of fluorescence between the two areas. The OCT system, OCS1300SS (Thorlabs Ltd.), was used to record the intensity of backscattered light of both areas. Multiple linear regression and paired t test were used to compare the change of the outcome measures. ***Results:*** All 3 instruments demonstrated significant dose responses with the erosive challenge interval ($p < 0.05$) and a detection threshold of 10 min from baseline. Thereafter, surface microhardness demonstrated significant changes after every 10 min of erosion, QLF at 4 erosive intervals (20, 40, 50 and 60 min) while OCT at only 2 (50 and 60 min). ***Conclusion:*** It can be concluded that OCT and QLF were able to detect demineralization after 10 min of erosive challenge and could be used to monitor the progression of demineralization of initial enamel erosion in vitro.

Dental erosion is defined as a chemical process that involves the dissolution of enamel and dentine by acid not derived from bacteria [Larsen, 1990]. The source of acid could either be extrinsic or intrinsic with extrinsic sources being mainly dietary and intrinsic ones being regurgitated gastric hydrochloric acid – often associated with gastro-oesophageal reflux disease. Depending on the aetiology, the recommended management of dental erosion includes behavioural and dietary modifications [Milosevic and O'Sullivan, 2008], consumption of modified acidic beverage [Bjelkhagen and Sundstrom, 1981] and the use of products that could increase the resistance to erosion.

In order to investigate the efficacy of any of the above management strategies, quantification of the degree of

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demineralization and longitudinal monitoring of the erosion lesion over the period of the therapy are important. To quantify and monitor initial erosion lesions, direct quantitative measurements of the degree of mineral loss with transverse microradiography [Amaechi et al., 1998] or indirect evaluations by assessing the degree of softening of initial eroded surface using surface microhardness had frequently been used. Nevertheless, the use of these techniques is limited to extra-oral assessments, hence the study designs of investigations using these techniques are restricted to in vitro or in situ. Dental erosion involves the interplay between biological, chemical and behavioural factors [Lussi and Hellwig, 2001; Lussi, 2006]. Even the most intricately designed in situ study fails to fully emulate the clinical environment especially of the interaction between the erosive and/or protective agent with saliva and the pellicle.

Hence evaluation techniques that can be applied in an in vivo setting could yield additional information that is currently scarce in the literature. Such clinical trials, as discussed by Huysmans et al. [2011], if conducted using healthy subjects have to be limited to inducing an early level of demineralization that is clinically insignificant and which could be reversed.

Fluorescence results from fluorophores in organic material that absorbs part of the light excitation energy and re-emits it at a lower energy. In the presence of demineralization, less fluorescence is observed. In vitro comparisons of fluorescence intensity with longitudinal microradiography have been performed on caries lesions [Hafstrom-Bjorkman et al., 1992; Al-Khateeb et al., 1997, 1998], natural incipient caries lesions [Emami et al., 1996] and advanced enamel erosion lesions [Pretty et al., 2004]. In these studies, strong correlations between amount of mineral loss and fluorescence loss have been found, ranging between 0.73 to 0.97.

Optical coherence tomography (OCT) is analogous to ultrasound imaging except that it uses light instead of sound waves. It uses a broad-band light source to perform cross-sectional imaging by measuring the magnitude and echo time delay of the backscattered light. OCT can be used to produce qualitative morphological cross-section images of near surface tissue structures and also quantitative measurements of the changes in the intensity of the backscattered light from different depths. In vivo imaging of hard and soft tissue has been demonstrated [Feldchtein et al., 1998], and it has also been used to monitor caries progression in orthodontic patients [Fried et al., 2007, 2010]. However, the use of OCT for dental erosion quantification has not been explored in vivo except with patients with gastro-oesophageal reflux disease, whose erosion rates were aggressive [Wilder-Smith et al., 2009]. Information on the sensitivity of OCT to small demineralization challenges in initial erosion lesions is still lacking.

Both OCT and quantitative light-induced fluorescence (QLF) have the potential of being used in longitudinal erosion studies in vivo. Before employing these techniques in clinical settings, it is necessary to first establish in vitro how sensitive these techniques are in quantifying demineralization of initial enamel erosion longitudinally. It is also important that the validation be undertaken on natural, unpolished enamel surface.

The aim of this study was to determine whether the progression of initial enamel erosion can be detected using OCT and QLF in vitro and also to determine their detection threshold for initial enamel erosion lesions.

Materials and Methods

The current study model was designed for the clinical reproduction of the initial stages of dental erosion, which involves surface-softening with no evidence of surface loss.

Sample Preparation and Erosion Cycle

Twelve extracted upper or lower human central incisors, caries free with no visually obvious fluorosis, were used in this study. The teeth were collected by the Kerala Dental School and were stored in thymol before the start of the study. Each tooth was fully embedded in separate cold-cured methylmethacrylate resin blocks with a dimension of 10 mm × 10 mm × 30 mm except for a 5 mm × 5 mm window at the middle third of its labial surface. The incisors were mounted such that the exposed labial surface was at least level with or not more than 1 mm higher than the adjacent resin. This was to ensure optimum contact with the orange juice and to keep the height variation consistent with the depth of field of QLF. One half of the exposed labial surface was protected with a non-residue masking tape while the other half was being coated with a one-step self-etch adhesive, Xeno® V (Dentsply), according to the manufacturer's instruction. This adhesive-coated area served as the reference area. After the application of the Xeno V had been completed, the protecting masking tape was removed from the first half for exposure to the erosive challenge. Baseline QLF and OCT images were captured and baseline surface microhardness measurements recorded.

The teeth mounted in the resin blocks were then suspended with plastic rods into a commercially available orange juice (ASDA orange juice from concentrate; pH 3.8 ± 0.1). Two groups of 6 teeth were suspended each in 500 ml of orange juice and the orange juice was gently stirred with a magnetic stirrer. The pH of the orange juice was monitored with a pH meter throughout the erosion cycle. After every 10 min for up to a total of 60 min, the teeth were removed from the orange juice and rinsed under a reservoir of running water for 1 min to remove excess acid from the surface of the teeth. The samples were then dried for 20 s with compressed air that is fixed 10 cm away from the resin block, and measurements

were taken with all three instruments. The orange juice was changed after every 10 min of erosive challenge interval.

Surface Microhardness

A Knoop micro-indenter (Microhardness Tester FM-700; Future-Tech Corporation, Japan) was used to assess the surface hardness of the tooth samples in this study. The resin blocks were placed flat on the translation stage and fixed at a reproducible position with the precision vice of the micro-indenter. A surface area of approximately 1 mm × 1 mm perpendicular to the direction of the load of the indenter was identified at the uncoated half of the enamel window for indentation. The identification of the perpendicular area was done by ensuring that the aiming beam of the micro-indenter was in focus before the loading was performed. Micro-indents were made using a Knoop diamond indenter, with a load of 25 g applied for 5 s.

Five indentations approximately 100 μm apart were made during each measurement time point at the identified area. The horizontal lengths were measured, and the Knoop hardness numbers *(KHN)* were calculated and averaged. Although care was taken to ensure that indentations were done on a surface that was perpendicular to the direction of loading, due to the inherent curvature of a natural and unpolished enamel surface and the presence of local irregularities such as perikymata, some asymmetrical indents were observed and were not included in the analysis. In these cases, additional indents were made. The outcome measure was expressed as the percentage of surface microhardness change *(ΔSMC)*, calculated based on the differences between KHN at baseline, $KHN(t_0)$ and the subsequent erosion intervals, $KHN(t)$. ΔSMC was calculated as:

$$\Delta SMC(t) = 100\left[\frac{KHN(t) - KHN(t_0)}{KHN(t_0)}\right].$$

Quantitative Light-Induced Fluorescence

A QLF imaging system was set up based on a custom-made ring-designed 395-nm LED array illumination (B5–437-CVD, Roithner Lasertechnik GmbH, Austria) to ensure uniform illumination over the sample. A blue bandpass filter centred at 425 nm was fitted in front of the array to prevent any light above 450 nm emitted from the LED to reach the sample. Images were captured with a triple charge-coupled device colour camera of 1,014 × 768 pixel resolution (HV-F31, Hitachi Kukasai Electric UK Ltd.). A 50-mm focal length-imaging lens (Fujinon HF50HA-1B, Fujifilm UK Ltd.) with a 10-mm extension was attached to the camera and a 515-nm long-pass yellow filter (OG-515, Edmund Optics Ltd., UK) was used to filter the fluorescence light emitted by the sample.

The images were taken in a dark enclosure and captured with bespoke software which enabled video repositioning of the samples at the various measuring time points with respect to baseline. An outline of the baseline image in the form of an overlay was superimposed on the live video of the image to facilitate alignment before an image was captured. Optimum light intensities and camera settings were determined to ensure that the fluorescence images from the sample were within the detection dynamic range of the camera and of maximum contrast.

A program was written in MATLAB (MathWorks Inc., USA) to analyse the images. Images from each specimen at the various erosion intervals were aligned so that representative regions of interest of the same location and dimension throughout the erosion intervals for the exposed and non-exposed area could be drawn.

The mean pixel value of the green channel was obtained for the defined regions of interest for the exposed, $F_E(t)$, and non-exposed areas, $F_{NE}(t)$. All images were analysed blind to the erosive challenge intervals, t. The percentage loss of fluorescence of the exposed area at each erosion interval, $\Delta F(t)$, was calculated as follows:

$$\Delta F(t) = 100\left[\frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)}\right],$$

where t_0 is the baseline time point.

Optical Coherence Tomography

A commercially available OCT system (OCS1300SS, Thorlabs Ltd., UK) was used to capture cross-sectional images of the exposed window of the tooth surface. The instrument incorporates a broad-band, frequency-swept laser source centred at 1,325 μm. The axial and transverse resolutions are of 9 and 15 μm in air, respectively, according to the manufacturer. The probing head was mounted with the beam facing downwards. The samples were placed on a translational stage perpendicular to the probe. The stage was fixed with a repositioning jig that enabled the sample to be repositioned to the same position and alignment during the different measuring time points.

The Thorlabs OCT capturing software (Swept Source OCT Imaging System Version 2.3.1, Thorlabs) was used to capture the image and control the OCT settings and guiding light beam. The light beam was configured to scan a length of 5 mm at the mesiodistal direction (x-axis) of the labial surface of the sample and at an axial depth of 3 mm. The incisocervical position of the light beam on the tooth surface (y-axis) was located at a cross-section with the least observed specular reflection. The (x, y) coordinate of the light beam for each sample was recorded for replication at consecutive measuring time points. The distance of the tooth surface to the probe was determined with the most convex area of the labial surface of the tooth at 1.0 ± 0.1 mm from the top of the displayed B scan.

A program was written in MATLAB (MathWorks Inc.) to load the OCT B scan images and analyse the changes of the backscattered light intensity in time. The B scans of each sample from the different measuring time points were aligned, and a similar region of interest was selected for all seven measuring time points. This region of interest consisted of 150 A scans (equivalent to a width of 1.35 mm) in each of the exposed and non-exposed areas. The curvature of the tooth surface in the selected region of interest was compensated by aligning the peak of the backscattered intensity rise occurring at the enamel-air interface in each A scan along the same horizontal pixel line of the B scan. A mean A scan curve was then generated from the selected window in the B scan for both the exposed and non-exposed areas.

The outcome measure for OCT was expressed as the mean percentage difference of decay in backscattered intensity, ΔD, between the exposed and non-exposed areas. The decay in backscattered intensity, D, is the relationship of the attenuation of backscattered light between two optical depths of an OCT A scan and is represented by the function below:

$$D = \frac{I_{plateau}}{I_{superficial}}.$$

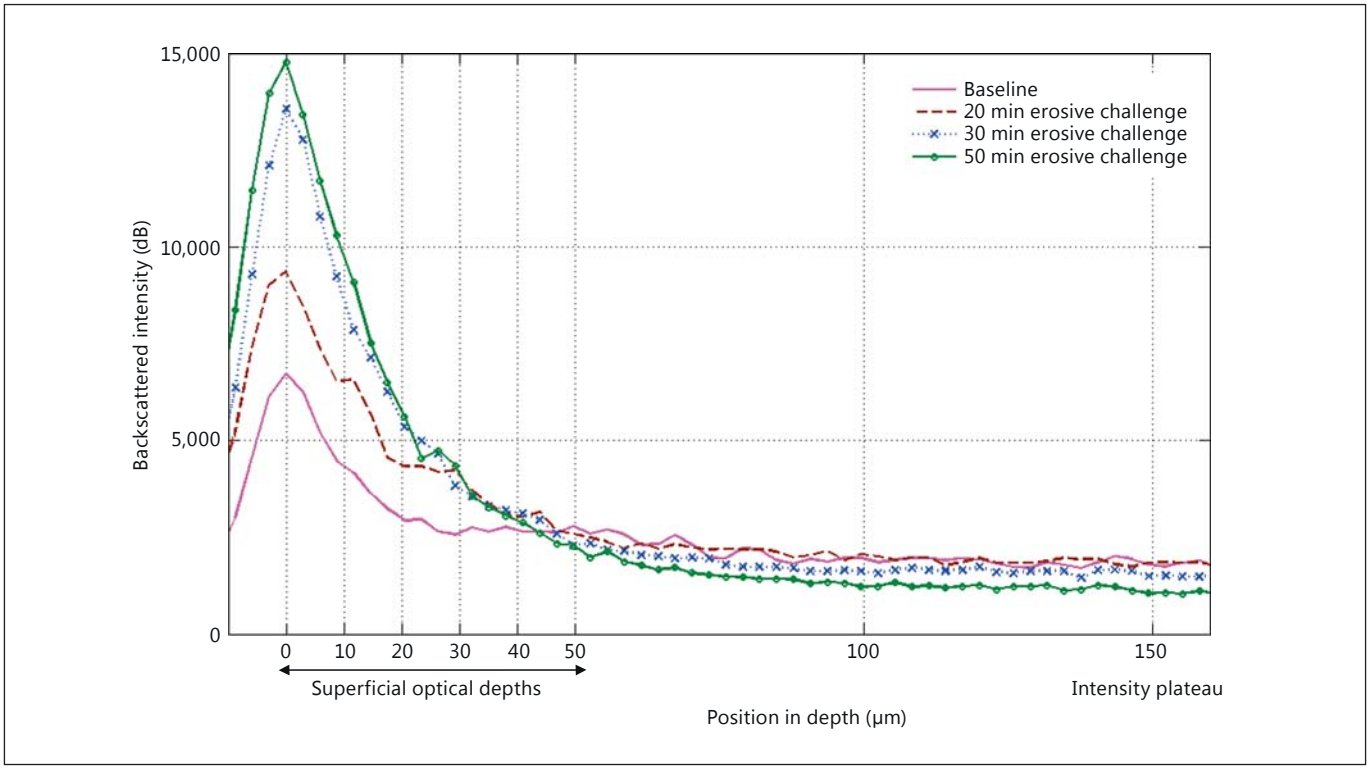

Fig. 1. Mean A scans of the exposed side of a sample at baseline, 20, 30 and 50 min of erosive challenge. The optical depths chosen for the study were 0, 10, 20, 30, 40 and 50 μm from the tooth-air interface. The observed optical depth for $I_{plateau}$ where the backscattered intensity reached a plateau was 150 μm.

$I_{superficial}$ is the intensity of backscattered light at or immediately below the tooth-air interface where demineralisation had occurred. The chosen superficial optical depths for this study were 0 μm (tooth-air interface), 10, 20, 30, 40 and 50 μm below the tooth-air interface (fig. 1). With the refractive index of enamel being 1.63, these chosen levels translated to the physical depth of 0, 6.25, 12.5, 18.75, 25 and 31.25 μm below the tooth-air interface, respectively. $I_{plateau}$ is the intensity of the backscattered light where the A scans had reached a plateau and were assumed not to be affected by the erosive challenge. It was observed to be at the optical depth of 150 μm for this study (fig. 1).

$\Delta D(t)$ at each measurement time point, t, is represented by the function below:

$$\Delta D(t) = 100\left[\frac{D_E(t)}{D_E(t_0)} - \frac{D_{NE}(t)}{D_{NE}(t_0)}\right],$$

where D_E is D of the exposed area, D_{NE} is D of the non-exposed area, t_0 is the baseline time point and t is the erosive challenge time point.

Statistical Analysis

The STATA™ 10.1 (Statacorp, Tex., USA) statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether time-related changes were detected with the 3 instruments.

When a significant time-related change was found for an outcome measure, a paired t test was performed to identify the detection sensitivity, which is defined as the shortest subsequent time intervals when a significant difference was detected.

Results

All the outcome measures were expressed in percentages of the non-exposed area, to account for biological variations between different teeth and systematic instrumental variation. The outcome measures of all 3 instruments were found to be normally distributed.

Surface Microhardness

Multiple regression analysis of ΔSMC with erosion interval showed that surface microhardness detected a significant erosion-interval-related decrease in microhardness with an approximately 80% reduction by the end of the 60-min erosion cumulative challenge. A linear func-

Table 1. Detection sensitivity of surface microhardness, QLF and OCT

Outcome measure	10 min	20 min	30 min	40 min	50 min	60 min
ΔSMC	22.72±0.05 (p = 0.001)	17.57±0.05 (p = 0.008)	15.07±0.05 (p = 0.016)	10.46±0.03 (p = 0.006)	9.07±0.03 (p = 0.017)	6.50±1.9 (p = 0.007)
ΔF	1.32±0.50 (p = 0.023)	2.44±0.70 (p = 0.001)		1.14±0.34 (p = 0.013)		2.15±0.45 (p = 0.001)
ΔD (150/20 μm)	15.6±5.82 (p = 0.023)		18.75±8.00 (p = 0.024)			
ΔD (150/30 μm)	18.42±6.03 (p = 0.005)	17.52±5.5 (p = 0.01)				8.9±3.8 (p = 0.049)
ΔD (150/40 μm)	12.17±3.2 (p = 0.026)		18.51±4.76 (p = 0.034)		12.50±4.85 (p = 0.028)	
ΔD (150/50 μm)	17.30±5.47 (p = 0.005)			15.07±5.57 (p = 0.029)		

Result of a paired t test between each consecutive interval of erosion for *ΔD* of various depth combinations, *ΔSMC* and *ΔF*. Only changes that were significantly different ($p < 0.05$) were recorded in this table.

tion can be fitted to it with an R^2 value of 0.810 ($p < 0.05$). The graph in figure 2 shows the mean *ΔSMC* for each erosion interval. As the pattern of its progression does not fully follow a linear function, the exponential and power functions were explored, and it was found that the correlation coefficients were similar to that of a linear function.

Paired t tests were used to analyse *ΔSMC* between each consecutive interval of erosion. Significant decreases in surface microhardness ($p < 0.05$) were detected between every 10 min of erosive challenge. There was approximately a 40% decrease in surface microhardness for the first 2 erosion intervals but a tapering of the decrease was found thereafter. At the 10-min erosion time point, the *ΔSMC* from baseline was 23.8 ± 5.5% (mean ± standard error, SE; $p = 0.001$) while at 60 min of erosion, the percentage change of the surface microhardness from the preceding erosion interval was 5.64 ± 1.9% (mean ± SE; $p = 0.007$) (table 1).

Quantitative Light-Induced Fluorescence

Multiple regression analysis of *ΔF* with erosion interval showed that QLF detected a significant erosion-interval-related loss of fluorescence. A linear function can be fitted to it with an R^2 value of 0.590 ($p < 0.05$). The graph in figure 3 shows the mean loss of fluorescence with erosion interval. It was found that there was 1.28 ± 0.55% (mean ± SE) loss of fluorescence during the first 10 min of erosion and cumulatively 8.90 ± 0.83% (mean ± SE) fluorescence loss at the end of 60 min of erosion.

Paired t tests were used to analyse the differences of the loss of fluorescence between each consecutive interval of erosion. Significant differences ($p < 0.05$) were first detected between baseline and 10 min of erosion and thereafter every consecutive 20 min of erosive challenge (10–30, and 30–50 min) and also the last 10 min of erosion (table 1).

Table 2. Result of multiple linear regression analysis of *ΔD* with erosive challenge interval

Superficial optical depth, μm	Plateau optical depth, μm	*ΔD*	
		R^2	p value
0	150	0.021	0.195
10	150	0.040	0.101
20	150	0.172	0.001
30	150	0.312	<0.000
40	150	0.305	<0.000
50	150	0.319	<0.000

Multiple linear regression analysis of *ΔD* for all 6 depth combinations with erosion interval. *ΔD* (150/20 μm), *ΔD* (150/30 μm), *ΔD* (150/40 μm) and *ΔD* (150/50 μm) showed significant erosion-related changes while *ΔD* (150/0 μm) and *ΔD* (150/10 μm) did not.

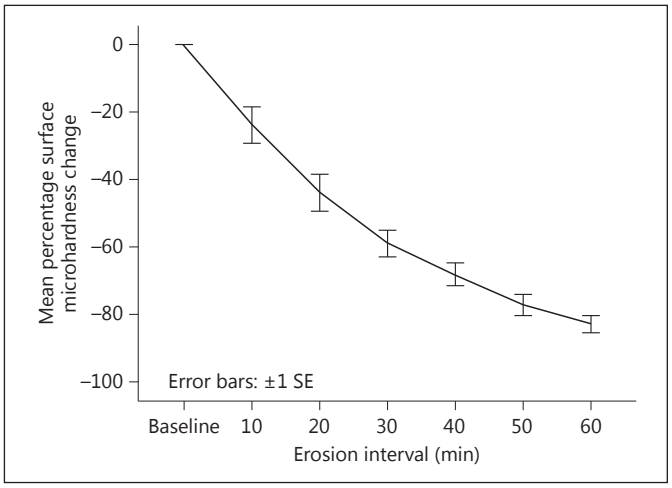

Fig. 2. Decrease in percentage change of surface microhardness *(ΔSMC)* as the erosion interval progressed.

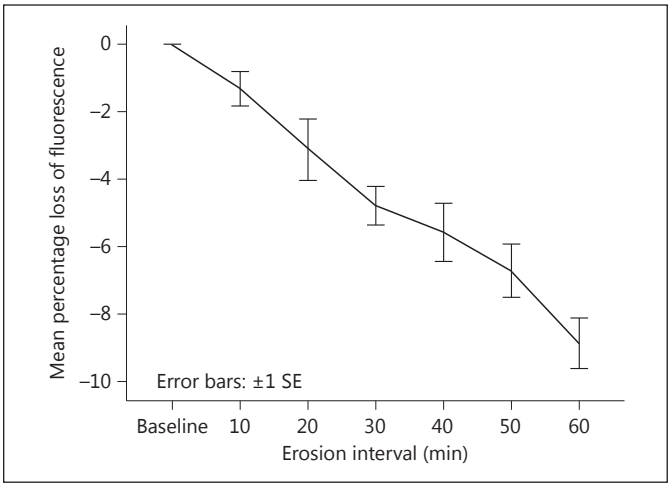

Fig. 3. Mean percentage loss of fluorescence *(ΔF)* as the erosion interval progressed.

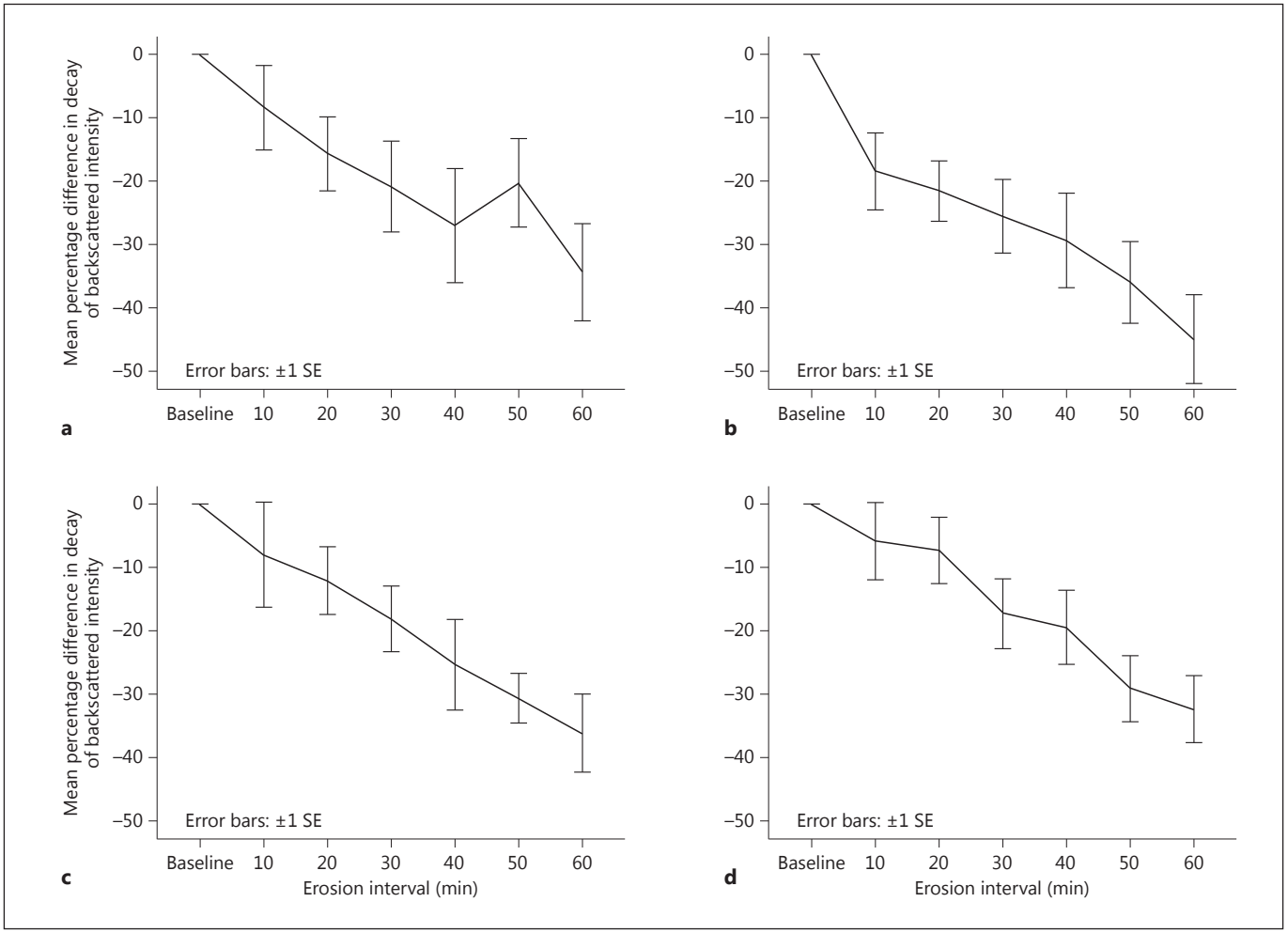

Fig. 4. Mean percentage difference of decay of backscattered intensity between exposed and non-exposed areas *(ΔD)*, for the depth combinations of 150/20 μm (**a**), 150/30 μm (**b**), 150/40 μm (**c**) and 150/50 μm (**d**) as the erosion interval progressed.

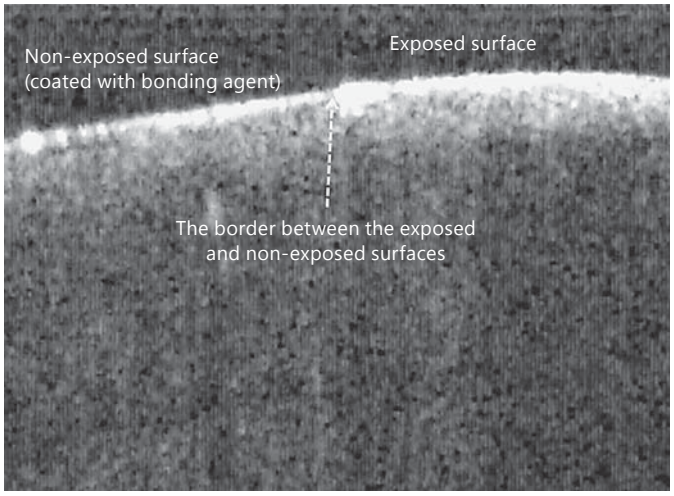

Fig. 5. OCT B scan of a sample after 60 min of erosive challenge. The arrow points to the border of the exposed and non-exposed surfaces. There is no discernible step change between the two surfaces (based on the axial resolution of the OCT of 15 μm).

Optical Coherence Tomography

Multiple linear regression analysis showed that there were significant erosion-interval-related changes in *ΔD* for some of the depth combinations as shown in table 2. *ΔD* (150/50 μm) demonstrated the highest R^2 value of 0.319, followed by *ΔD* (150/30 μm) with a value of 0.312.

Figure 4 illustrates the progression of *ΔD* throughout the erosive challenge for the various depth combinations that showed significant erosion-interval-related changes. *ΔD* for the depth combinations of 150/20, 150/30, 150/40 and 150/50 μm demonstrated a gradual decrease as erosive challenge progresses and at the end of the 60-min erosive challenge, *ΔD* for these depth combinations decreased 30–40%. Half of this decrease occurred early during the first 10 min for the depth combinations of 150/20 and 150/30 μm while it occurred between 10 and 20 min for the depth combinations of 150/40 and 150/50 μm.

Paired t tests were also performed to evaluate the detection sensitivity of these 4 optical depth combinations, and the results are presented in table 1. The *ΔD* for depth combinations of 150/30 and 150/40 μm both showed significant differences in decay at 3 time points. *ΔD* of 150/30 μm demonstrated early detection sensitivity at 10 min but subsequently only at 50 and 60 min of erosive challenge. *ΔD* for 150/40 μm demonstrated a significant difference in decay at every 20-min interval. 150/20 and 150/50 μm showed significant differences in decay at only 2 time points and of longer intervals.

Discussion

The current study model was designed to simulate the clinical stages of initial enamel erosion. The degree of demineralization incurred in the present study was small, only involving surface-softening with no evidence of surface loss or step change of more than 10 μm as observed in the B scans of OCT at the end of the erosion interval (fig. 5).

Surface Microhardness

Featherstone et al. [1983] demonstrated a direct relationship between volume percent mineral and the square root of the Knoop hardness values for 40–90% demineralized dental enamel and concluded that although microhardness and microradiographic profiles measure different physical properties, the two are closely interlinked and microhardness profiles can therefore be used not only as a comparative measure of hardness changes, but also as a direct measure of mineral loss as a consequence of demineralization. Hara and Zero [2008] and Jaeggi and Lussi [1999] had also shown that surface microhardness is sensitive for detecting the initial stages of erosions with softening of the enamel surface but has limitations in the analysis of advanced lesions with substance loss. Therefore surface microhardness was chosen as the established assessment technique for initial erosion by which QLF and OCT were compared to in this study.

The use of flat, polished surfaces has been recommended to produce well-defined indentations but in order to satisfy the objective of this study of validating the use of QLF and OCT for the end purpose of application in clinical trials, natural, unpolished labial surfaces were used. Caldwell et al. [1957] first described the measurement of surface microhardness on an intact surface of enamel. They found that after excluding asymmetrical indentations, the variation in hardness of intact surfaces is usually the result of local differences in hardness of the intact surfaces and not attributable solely to mechanical difficulties in obtaining symmetrical indentations.

The labial surfaces of upper and lower central incisors were chosen for this study due to their relatively flat profile. The mean KHN for this study ranged from 306.5 ± 24.8 at baseline to 54.44 ± 7.67 after 60 min of erosive challenge in orange juice. The standard deviations of the indentations measured were comparable to those of polished flat surfaces [Marillac et al., 2008]. In order to limit the impact of surrounding material changes, Featherstone et al. [1983] and Lussi et al. [1995] suggested that micro-indentations are to be performed with low pres-

sure of not more than 50 g. The load of 25 g with a dwelling time of 5 s was chosen for this study as it is the smallest load with the shortest loading time needed to produce a detectable indent under magnifications of ×50 for all the 12 samples at baseline. This was especially important when specimens with a natural surface were used. To start with the smallest detectable indent would mean it would be less likely to end up with large indents that surpass the dimension of a flat plane present on the slightly curved surface of a central incisor.

Quantitative Light-Induced Fluorescence

A customized QLF set-up was used in this study to optimize the field of view for the size of the specimens used. The level of fluorescence loss of this study is similar to that of Ablal et al. [2009] and Pretty et al. [2004] despite different types of samples and QLF system used. Ablal et al. [2009] subjected polished bovine incisors to orange juice and there was approximately a 2% loss of fluorescence at 20 min of erosive challenge with orange juice. Pretty et al. [2004] on the other hand demonstrated approximately a 2% loss of fluorescence at 30 min of erosive challenge with 0.1% citric acid on the natural surface of extracted human premolars. As shown in figure 3 of this study, there was 3.14 ± 0.91% (mean ± SE) of mean fluorescence loss after 20 min of erosive challenge with orange juice.

Optical Coherence Tomography

At the enamel-air interface of OCT images, specular reflection is very strong and it could be around 20 dB (100 times) higher than the backscattering intensity and could therefore mask any information about scattering at or just below the tooth surface [Fried et al., 2002]. This strong specular signal could lead to residual coherence artefacts, spikes or 'columnar' artefacts in the image. Amaechi et al. [2001] in their attempt to minimize the confounding effect of specular reflection during the quantification of early artificial caries described the exclusion of varied distances from the surface for different specimens to serve as cut-off depths. As the patterns of demineralization of in vitro induced erosion lesions are generally more homogeneous and uniform than caries and with ΔD of the various combination depths, a more systematic way of ascertaining a cut-off level for exclusion of the effect of specular reflection could be done in this study.

The combination depth of 150/0 and 150/10 μm did not show any time-related ΔD changes throughout the 60-min erosion intervals. This is most likely attributed to the masking effect of the intense specular reflection. The combination depth of 150/20 μm is the most superficial layer that demonstrated significant time-related changes. There was a gradual decrease in mean percentage difference in decay from baseline to 40 min of erosion but there was a sudden increase in mean percentage difference in decay at 50 min of erosion (fig. 4). This suggests that the backscattered intensity at 20 μm might still be confounded by surface specular reflectance though at a lesser magnitude. Therefore a statistical cut-off depth of 20 μm for specular reflection for this study was chosen.

The depth combination of 150/30 and 150/40 μm seemed to be the optimum depths to be used in the measuring of initial demineralization with OCT. The reasons are twofold. Firstly, these two depth combinations are the levels nearest to the tooth-air interface that showed significant linear regression with erosion interval but not affected by specular reflection. Secondly, both demonstrated significant difference in decay at 3 time points. For small erosive challenge, ΔD at 150/30 μm seemed to be the depth combination of choice as it detected a significant difference as early as 10 min of erosion but during the subsequent erosion challenges, it is not as sensitive as ΔD of 150/40 μm. ΔD of 150/40 μm detected significant differences uniformly at every 20 min of erosive challenge throughout the 60-min erosive challenge.

OCT however provides additional information of subsurface characteristics of the eroded enamel. It not only provides information about the degree of demineralization from the backscattered intensity, it also provides information about lesion depth. As demineralization progressed, the progression of the porosity from the enamel-air interface towards the deeper layers is indirectly mirrored by the chronological progression of the occurrence of significance of difference in decay from the more superficial layers of enamel to the deeper layers. This feature, which is not possible with QLF, could potentially be used to compare the severity of erosion between samples at one particular time point or in a cross-sectional study design. The OCT backscattered signal is not confounded by discolouration either – an issue that frequently affects QLF. A discoloured area viewed with light-induced fluorescence appears dark like a demineralized area. This is due to the absorption of light of the discoloured area [van der Veen and de Josselin de Jong, 2000].

The lower correlation of OCT with erosion interval and surface microhardness could be attributed to the larger standard deviations of the OCT measurements. These larger standard deviations could be due to a combination of deviations in repositioning between the various measurement time points and the residual confound-

ing effect of specular reflection. In this study the OCT measurements are more susceptible to deviations in repositioning than QLF as only one cross-section (B scan) was acquired while the QLF measurements were from an area. This repositioning problem encountered with OCT could potentially be minimized by taking 3-dimensional OCT images.

In conclusion, a significant positive correlation between the outcome measure of both QLF and OCT with erosion interval was found. Nevertheless, within the limits of the current design, QLF seemed to be a better diagnostic tool than OCT in the detection and monitoring of demineralization as it demonstrated a higher correlation with erosion intervals.

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Disclosure Statement

There is no conflict of interest for any of the authors that might introduce bias or affect their judgement.

References

Ablal MA, Kaur JS, Cooper L, Jarad FD, Milosevic A, Higham SM, Preston AJ: The erosive potential of some alcopops using bovine enamel: an in vitro study. J Dent 2009;37:835–839.

Al-Khateeb S, Forsberg CM, de Josselin de Jong E, Angmar-Mansson B: A longitudinal laser fluorescence study of white spot lesions in orthodontic patients. Am J Orthod Dentofacial Orthop 1998;113:595–602.

Al-Khateeb S, ten Cate JM, Angmar-Mansson B, de Josselin de Jong E, Sundstrom G, Exterkate RA, Oliveby A: Quantification of formation and remineralization of artificial enamel lesions with a new portable fluorescence device. Adv Dent Res 1997;11:502–506.

Amaechi BT, Higham SM, Edgar WM: Use of transverse microradiography to quantify mineral loss by erosion in bovine enamel. Caries Res 1998;32:351–356.

Amaechi BT, Higham SM, Podoleanu AG, Rogers JA, Jackson DA: Use of optical coherence tomography for assessment of dental caries: quantitative procedure. J Oral Rehabil 2001; 28:1092–1093.

Bjelkhagen H, Sundstrom F: A clinically applicable laser luminescence method for the early detection of dental-caries. IEEE J Quantum Elect 1981;17:226–228.

Caldwell RC, Muntz ML, Gilmore RW, Pigman W: Microhardness studies of intact surface enamel. J Dent Res 1957;36:732–738.

Emami Z, al-Khateeb S, de Josselin de Jong E, Sundstrom F, Trollsas K, Angmar-Mansson B: Mineral loss in incipient caries lesions quantified with laser fluorescence and longitudinal microradiography. A methodologic study. Acta Odontol Scand 1996;54:8–13.

Featherstone JD, ten Cate JM, Shariati M, Arends J: Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 1983;17:385–391.

Feldchtein F, Gelikonov V, Iksanov R, Gelikonov G, Kuranov R, Sergeev A, Gladkova N, Ourutina M, Reitze D, Warren J: In vivo OCT imaging of hard and soft tissue of the oral cavity. Opt Express 1998;3:239–250.

Fried D, Louie T, Lee C, Hsu D, Hirasuna K, Manesh S, Staninec M, Darling CL: Clinical assessment of early tooth demineralization using polarization sensitive optical coherence tomography. Lasers Surg Med 2010;42:738–745.

Fried D, Ngaotheppitak P, Darling CL, Ho CM: Polarization sensitive optical coherence tomography for quantifying the severity of natural caries lesions on occlusal surfaces – article No 64250u. Lasers Dent XIII 2007; 6425:U4250.

Fried D, Xie J, Shafi S, Featherstone JD, Breunig TM, Le C: Imaging caries lesions and lesion progression with polarization sensitive optical coherence tomography. J Biomed Opt 2002;7:618–627.

Hafstrom-Bjorkman U, Sundstrom F, de Josselin de Jong E, Oliveby A, Angmar-Mansson B: Comparison of laser fluorescence and longitudinal microradiography for quantitative assessment of in vitro enamel caries. Caries Res 1992;26:241–247.

Hara AT, Zero DT: Analysis of the erosive potential of calcium-containing acidic beverages. Eur J Oral Sci 2008;116:60–65.

Jaeggi T, Lussi A: Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. Caries Res 1999;33: 455–461.

Huysmans MC, Chew HP, Ellwood RP: Clinical studies of dental erosion and erosive wear. Caries Res 2011;45(suppl 1):60–68.

Larsen MJ: Chemical events during tooth dissolution. J Dent Res 1990;69(Spec No):575–580, discussion 634–636.

Lussi A: Erosive tooth wear – a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 2006;20:1–8.

Lussi A, Hellwig E: Erosive potential of oral care products. Caries Res 2001;35(suppl 1):52–56.

Lussi A, Jaeggi T, Jaeggi-Scharer S: Prediction of the erosive potential of some beverages. Caries Res 1995;29:349–354.

Marillac MDS, Delbem AC, Vieira RS: Effect of time in hardness test on artificially demineralised human dental enamel. Braz J Oral Sci 2008;7:1507–1511.

Milosevic A, O'Sullivan E: Diagnosis, prevention and management of dental erosion: summary of an updated national guideline. Prim Dent Care 2008;15:11–12.

Pretty IA, Edgar WM, Higham SM: The validation of quantitative light-induced fluorescence to quantify acid erosion of human enamel. Arch Oral Biol 2004;49:285–294.

Van der Veen MH, de Josselin de Jong E: Application of quantitative light-induced fluorescence for assessing early caries lesions. Monogr Oral Sci 2000;17:144–162.

Wilder-Smith CH, Wilder-Smith P, Kawakami-Wong H, Voronets J, Osann K, Lussi A: Quantification of dental erosions in patients with GERD using optical coherence tomography before and after double-blind, randomized treatment with esomeprazole or placebo. Am J Gastroenterol 2009;104:2788–2795.